Beyond BRCA: The Use of Homologous Recombination Deficiency Testing to Guide the Use of PARP Inhibitors

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Before we go beyond BRCA...

...let’s consider what was learned with BRACAnalysis™ CDx

What’s in the label?
gBRCA 1/2 mutation CDx: BRACAnalysis™

BRACAnalysis CDx™ is an *in vitro* diagnostic device intended for the qualitative detection and classification of variants in the protein coding regions and intron/exon boundaries of the *BRCA1* and *BRCA2* genes using genomic DNA obtained from whole blood.

Deleterious or suspected deleterious germline *BRCA* variants eligible for treatment with Lynparza™ (olaparib).

**Precedence setting**

- First LDT approved as a companion diagnostic through PMA
- Developed system for the classification and interpretation of all variants pre- and post-approval
Summary of Primary Clinical study

• Single-arm, open-label, multi-center study in patients with advanced cancers who have a deleterious or suspected deleterious germline BRCA mutation (gBRCAm) (n=317) (Basket clinical trial)

• Patients used for approval were a subset
  • Subset: 193 patients w/ deleterious or suspected deleterious gBRCAm-associated ovarian cancer
  • Subset: 137 had measurable disease and had received three or more lines of prior chemotherapy (matched indication)

• Clinical utility based on bridging, retrospective analysis
  • Archive samples from 61 (out of 137) patients were available for retrospective testing
  • The samples were tested in a blinded manner at the single site, and the only site approved in the US.
The result of a BRCA mutation

BRCA DNA repair function

- BRCA is critical to DNA repair through homologous recombination, a high fidelity repair process that is template directed

- Loss of BRCA function is associated with genomic scarring through Homologous recombination deficiency (HRD) and low fidelity DNA repair

Normal Karyotype

BRCA1mut ovarian cancer Karyotype
Germline BRCA1/2 mutations do not account for all of the HR deficient tumors in ovarian cancer

Molecular Profile of Primary High Grade Serous Ovarian Cancer
>50% of Tumors Characterized by Deficiencies in DNA Repair genes

Patient selection for agents that exploit homologous recombination deficiency?

Germline BRCA1/2 mutations do not account for all of the HR deficient tumors in ovarian cancer.

Molecular Profile of Primary High Grade Serous Ovarian Cancer
~50% of Tumors Characterized by Deficiencies in DNA Repair genes

- BRCA1 hypermethylation (11.5%)
- EMSY amplified/mutation (8%)
- Germline BRCA1 mutation (8%)
- Germline BRCA2 mutation (7%)
- PTEN deficiency (7%)
- FA complex mutation (5%)
- Somatic BRCA2 mutation (3%)
- Somatic BRCA1 mutation (3%)
- RAD51C hypermethylation (3%)
- ATM/ATR mutation (1%)
- unknown (56%)

Patient selection for agents that exploit homologous recombination deficiency?

Strategies for patient selection

HGSOC population at presentation

- **HR proficient**
  - gBRCAmut only
  - Multi-panel
  - HRD profile

- **HR deficient**
  - Likely responders to Platinum or PARP based therapies

**Germline BRCA1/2 mutation**
- Very well validated test
- Informs treatment for 15% of the HGS-OvCa population with 1 test

**Individual markers/ or Panel**
- Could inform treatment for 50% of the population but requires each marker be individually validated and used

**HRD score (including BRCA\text{mut})**
- Informs treatment for >50% of the HGS-OvCa population with 1 test

Selection of the MyChoice HRD test to identify likely PARPi (niraparib) responders and non-responders

- The HRD test is a next generation based sequencing assay
- It outputs the following things from tumor tissue
  - BRCA1 mutations
  - BRCA2 mutations
  - HRD score
- BRCA mutations are classified in the same manner as BRACAnalysis. Classification is a key issue for approval as a companion diagnostic
- The HRD test does not clarify origin of mutation (germline or somatic)
- The HRD score is based on genomic scarring driven by functional defects in BRCA1/2 and other genes
- It is based on sequencing ~54,000 SNP (Agilent Sure Select) regions of the genome and the BRCA1/2 genes – total of 21Mb sequence data (HiSeq)
- HRD has been shown to be a predictor of Platinum response

The myChoice HRD™ assay is currently in clinical development by Myriad Genetics Laboratories
Homologous Recombination (HR) vs Non-Homologous End Joining (NHEJ)

**Homologous Recombination**
- Double strand break
- High-Fidelity repair
- Loss of heterozygosity: LOH
- Telomeric Allelic Imbalance: TAI
- Large-scale State Transitions: LST
- Chromosomal breaks in adjacent regions > 10mb

**Non-homologous End Joining**
- Low-Fidelity repair can result in genomic instability that can be quantified
- Loss of heterozygosity > 15 mb
- Allelic imbalance extending to the telomere
- Mitosis
Components of the HRD score

**HRD Score**: The sum of the LOH+TAI+LST scores (0-100)

**LOH Score**: The number of LOH regions longer than 15 Mb but shorter than the length of a whole chromosome

**TAI Score**: The number of regions with allelic imbalance which extend to the subtelomere but do not cross the centromere

**LST Score**: The number of chromosomal breaks between adjacent regions longer than 10 Mb after filtering out regions shorter than 3 Mb

Example of a genomic profile
Ovarian tumor with low HRD score (HRD = 16)

Green dots represent normalized sequence reads counts for each SNP. Blue dots represent allele dosage (proportion of sequence reads representing one allele of a SNP) for each SNP. Red lines represent reconstructed copy number. Yellow lines represent reconstructed lowest allele-specific copy number. LOH corresponds to lowest allele specific copy number zero. Examples of LOH region, TAI region, and LST break points are shown in the sample with low HRD score.
MyChoice™ HRD Test Can Discriminate High vs. Low HRD Tumors

**Example of a Genomic Profile of Low HRD Tumor (HRD = 16)**

**Example of a Genomic Profile of High HRD Tumor (HRD = 81)**

**HRD Distribution of 561 Ovarian Tumors:**
BRCA Deficient Tumors Include Germ Line, Somatic and BRCA Promoter Methylated

**Cutoff of ≥42 Captures 95% BRCAdef**

Biology defines the cutoff
A combination of three scores of genomic instability separates HRD+ and HRD- tumors

- HGSOC has two distinct tumor types: HR proficient and deficient (i.e. BRCA deficient).
- 3 Individual measures (scores) of instability have been developed.
- The sum of scores resolves the two populations better than any individual score alone.

Analysis conducted on 561 ovarian tumor samples,
Niraparib: Advanced PARP inhibitor (multiple phase 3 studies)

- Orally active, potent PARP 1/2 inhibitor
- Clinical and genetic enrichment allows for targeted development
- myChoice HRD® Test enables clear determination of HRD patients in breast and ovarian cancer

Adapted from: Walsh et al, Gyn.Onc., 137 (2015), 343-350
Building pre-clinical validation of HRD for niraparib using patient derived xenografts

Orthotopic models

Responding “Sensitive”

Non-Responding “Resistant”
HRD Score Correlates with Niraparib Sensitivity

- PDX models were assessed for HRD score
- All tumors with a BRCA mutation had a HRD score >42
- Niraparib sensitive tumors included wild-type BRCA tumors with those with BRCA mutations
- All niraparib sensitive tumors had HRD scores >42

Ovarian and Breast PDX

- Sensitive to niraparib
- Resistant to niraparib

In collaboration with the Mayo Clinic (US), Pharmaron (CH), Xentech (FR) & Myriad HRD was calculated in primary patient/PDX models
The HRD Test Identifies Niraparib Sensitive Tumors

- In PDX models, Niraparib sensitivity can be defined with the a cut off of (>42)
- The response rate of BRCA WT tumors was similar to that of tumors with BRCA mutations

<table>
<thead>
<tr>
<th>Tumor with completed evaluation in vivo (3/10/2015)</th>
<th>Breast (n = 25)</th>
<th>Ovarian (n = 26)</th>
<th>Total (n = 51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive tumors/all tumors tested</td>
<td>8/25 (32%)</td>
<td>10/26 (38%)</td>
<td>18/51 (35%)</td>
</tr>
<tr>
<td>BRCAmut sensitive /BRCAmut tumors tested</td>
<td>3/6 (50%)</td>
<td>4/6 (67%)</td>
<td>7/12 (58%)</td>
</tr>
<tr>
<td>Sensitive tumors/HRD+ tumors tested</td>
<td>8/17 (47%)</td>
<td>10/17 (59%)</td>
<td>18/34 (53%)</td>
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</tbody>
</table>
Phase 3 Trial of Niraparib in 2nd Line (Recurrent) Ovarian Cancer Maintenance (NOVA Trial)

Ovarian cancer is initially treating by debulking and then platinum treatment

~80-90 of patients relapse

High Grade Serous Ovarian Cancer, Platinum Sensitive*, Relapsed

Response to Platinum Treatment
n=490

- gBRCA\textsuperscript{mut}
- Non-gBRCA\textsuperscript{mut}

2:1 Randomization

Niraparib 300 mg
n=120

Placebo
n=60

Niraparib 300 mg
n=207

Placebo
n=103

What does HRD look like in this population?

• Primary Endpoint: PFS
• Each cohort independently assess
• Prospective Retrospective analysis of HRD population hierarchically

* Platinum sensitivity is defined by ≥6 months PFS
Preliminary HRD Distribution in NOVA Tumors

HRD Distribution of 174 Tumors from Both Cohorts

- gBRCAmut cohort
- Non-gBRCAmut cohort

cutoff

Nearly all gBRCdef and tBRCAdedef Tumors Have High HRD Scores
### Applying HRD prospectively and retrospectively in Ovarian Cancer

#### 2nd Line (Recurrent) NOVA Trial
- PFS primary endpoint
- Enrollment complete
- Efficacy analyses in prespecified gBRCA\textsuperscript{mut} and non-gBRCA/HRD+ patients
- Assignment of HRD+ status expected in Q4 2015

#### Ovarian 4th line+ QUADRA Trial
- ORR primary endpoint
- Efficacy analyses in prespecified gBRCA\textsuperscript{mut} and HRD+ subgroups
- Phase 2 trial ongoing
- Initial data expected in early 2016

#### 1st Line PRIMA Trial
- PFS primary endpoint
- Will enroll HRD+ patients including gBRCA\textsuperscript{mut}
- Phase 3 trial to begin Q4 2015

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**Other studies utilizing HRD prospectively and retrospectively are currently in planning**
BRCA testing as a CDx was precedence setting
HRD biology results in a “scarring of chromosomes”
HRD “scar” can be quantified
HRD+ cut off was defined based on biology, independent of therapy
HRD biology correlates with PARPi sensitivity in PDX models
HRD biomarker has been introduced into niraparib studies
  • NOVA: prospective analysis of non-gBRCA subgroup
  • QUADRA: 4th line treatment trial (ORR and duration)
  • PRIMA: selection of HRD+ patients
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